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Cost analysis of *Agave potatorum* Zucc, produced *in vitro* by direct organogenesis

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ABSTRACT

Objective: To demonstrate the efficiency and profitability of temporary immersion bioreactors compared to propagation in gelled media.

Design/Methodology/Approach: *Agave potatorum* seedlings were introduced to the *in vitro* system in order to compare their productivity, multiplication rate, and propagation time. An investment project was carried out considering the equipment depreciation, but without considering the construction of the property. Based on these premises, the cost of production per plant was evaluated with a goal of 500,000 seedlings in mind.

Results: A 3% contamination was recorded when the *in vitro* system was first introduced. The semi-solid multiplication rate was 4 shoots per explant and 18 shoots per explant in bioreactors, both at 30 days of incubation. A 10% handling loss was taken into consideration. The production cost was US$0.16 (MNX$3.20) for gelled media and US$0.09 (MNX$1.80) for propagation in temporary immersion bioreactors. The Internal Rates of Return for gelled media and for propagation with bioreactors were 2.33 and 3.75, respectively.

Study Limitations/Implications: The study does not take into consideration the construction of the property, although it does consider equipment depreciation.

Findings/Conclusions: Thanks to scientifical and technological development, the use of biotechnological tools is becoming more profitable every day. These technologies are already available to be transferred and this kind of research demonstrates its profitability, highlighting the potential establishment of technology-based companies.

Keywords: Biofactory, agave used for mezcal, profitability, direct organogenesis.

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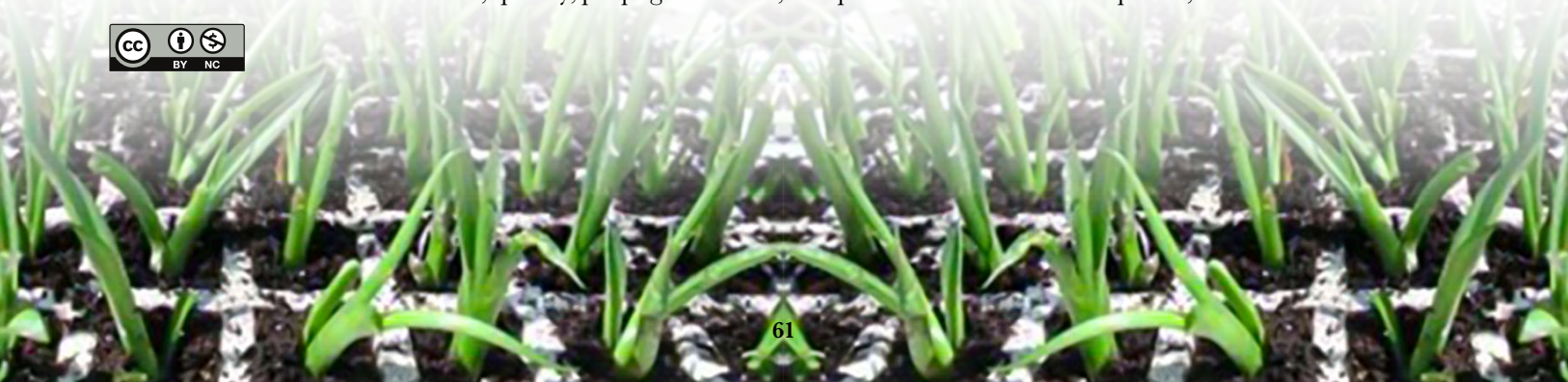
INTRODUCTION

All over the world, research-focused plant tissue culture laboratories have developed a series of protocols for the propagation of various plant species. Private laboratories (biofactories) use these protocols as a basis for massive plant propagation but consider parameters that research centers do not take into consideration —*e.g.*, the multiplication rate, quality, propagation time, and production costs of these plants, as well as the workforce



involved. Likewise, few studies have analyzed production costs and the profitability of these techniques in different plant species (Alamilla-Magaña *et al.*, 2019; Amine Marzri *et al.*, 2021; Criollo-Chan *et al.*, n.d.).

Tobalá spp. (*Agave potatorum* Zucc.) is used to produce mezcal and is currently threatened by unregulated use. These wild plants reproduce through seeds and its populations are being wiped out, along with their genetic variability, since, being non-orthodox plants, their seeds have a maximum viability of 24 months (Enríquez del Valle *et al.*, n.d.; Langlé Argüello *et al.*, November 30 and December 1; Pérez de León *et al.*, 2020).

The *in vitro* multiplication of *Agave potatorum* can be a tool to achieve the massive multiplication that the mezcal market is demanding and this methodology can also be used to repopulate wild plantations. Currently various authors report propagation protocols in semi-solid media with average rates ranging from 6.9 to 14.5 shoots per explant (Aguilar-Jiménez *et al.*, 2018; Domínguez Rosales *et al.*, 2008; Pérez De León, 2022). However, there are very few reports on the propagation of *A. potatorum* using temporary immersion bioreactors (TIB). The present study provides an estimation of the production costs of *Agave potatorum* by direct organogenesis using gelled media and liquid media with twin-flask type TIBs, designed by Escalona *et al.* (1999).

MATERIALS AND METHODS

Process description

The multiplication process by direct organogenesis is based on the use of Tobalá agave meristems and includes the following stages: induction of adventitious shoots, shoot multiplication, shoot elongation, and acclimatization. All experimental data were taken from Pérez De León (2022). Figure 1 shows the flow diagram of the direct organogenesis process.

Induction of adventitious shoots

The study began with fifty *Agave potatorum* meristems, which were subsequently multiplied to obtain the initial amount (500,000 seedlings) and to estimate the production costs (Table 1). Explant disinfection was performed as reported by Aguilar-Jiménez *et al.* (2018). The explants were washed with water and Tween 20 (Cat. 822184, Merck). Subsequently, the explants were put inside a laminar flow hood for 3 minutes in a 70% (v/v) ethanol solution. At the end of that period, they were immersed in a 10% aqueous solution of commercial sodium hypochlorite (Cloralex®) for 15 minutes. Finally, they were rinsed three times with sterile distilled water and the tender leaves covering the meristem were removed until an ≈1-cm explant was obtained. The resulting explants were seeded in a MS medium (Murashigue and Skoog, 1962, Cat. MSP01, Caisson Labs) —supplemented with 3% sucrose (Cat. S011, Caisson Labs) and 0.3% TC Gel (Cat.PTP02, Caisson Labs)— and incubated at 25±2 °C for 30 days with a 16h/8h light/dark photoperiod, in order to evaluate the sterility of the explants. Subsequently, contaminant-free explants were planted in a MS medium (Cat. MSP01, Caisson Labs) supplemented with 2 mg L^{-1} of benzylaminopurine (BA, Cat. B800, Phytotechnology) and 4% sucrose (Cat. S011, Caisson Labs).

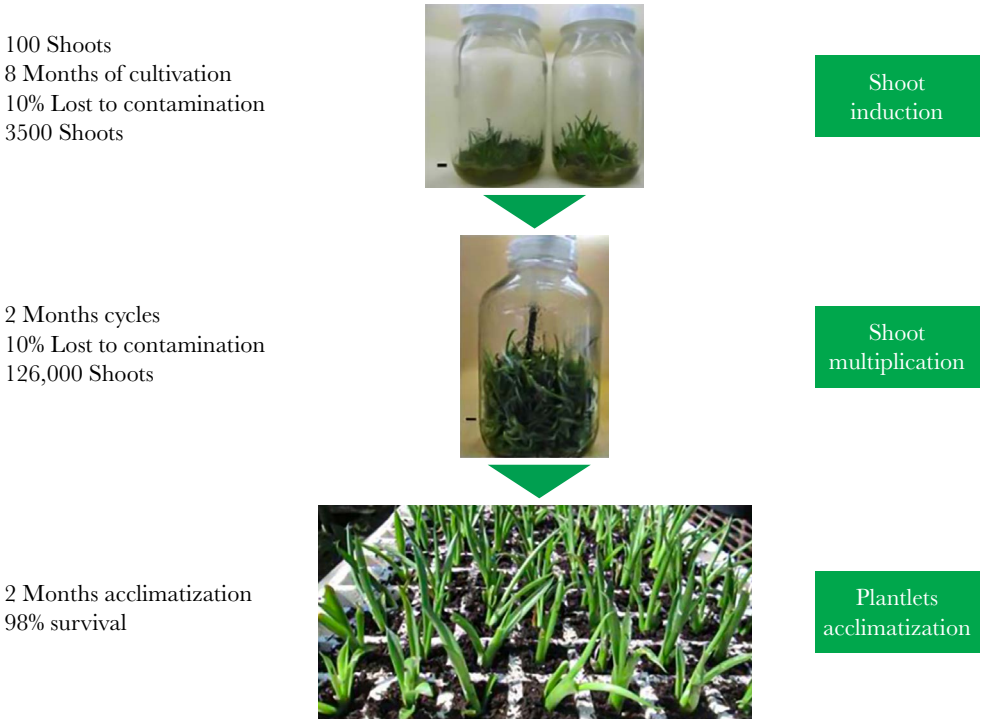


Figure 1. Flow diagram of the propagation process by direct organogenesis.

Table 1. Induction and multiplication process in production systems with gelled media and liquid media (temporary immersion bioreactors: TIB).

Induction		**Multiplication**		
SS		**PHASES**	**SS**	**TIB**
Initial meristems	100	Initial explants	17500	8000
Induction cycles, 60 days	5	Multiplication cycles	11	5
Explants obtained at the end	149,778	Final explants obtained	500,500	608,000

Adventitious shoots multiplication

Organogenic cultures were grown in a MS medium (Cat. MSP01, Caisson Labs) supplemented with 3% sucrose (Cat. S011, Caisson Labs), 10 mg L^{-1} BA (Cat. B800, Phytotechnology), and 0.3 mg L^{-1} indole acetic acid (IAA, Cat. I885, Phytotechnology). The pH was adjusted to 5.7 and it was sterilized in an autoclave at 120 °C, at 1 atm pressure for 20 min. They were incubated at 25±2 °C for 30 days with a 16h/8h light/darkness photoperiod, at 32 μmol s^{-1} m^{-2}. Shoots longer than 4.0 cm, with at least three leaves, were chose for acclimatization.

Seedling acclimatization

The acclimatized seedlings had at least three leaves and were at least 10 cm long. They were cultivated in polystyrene trays with 200 cavities, with a substrate made of a 50:50 mix of peat moss (Sushine mix #3) and greenhouse soil. They were placed in a shade-house

with 50% shade cloth for 60 days at room temperature. The first seven days, the seedlings were irrigated every day with 50-L/min sprinklers for 30 min; subsequently, they were irrigated every two days under the same conditions.

Cost Calculation

At the beginning of the multiplication, the induction process, and the work to obtain the required number of shoots took 5 months. A goal of 500,000 seedlings per year was established; therefore, the TIB was carried out in 5 cycles of 30 days and, in the case of semi-solid media, it was carried out in a 11-month period. The components considered in the cost analysis are included in the Calculation Report. A Caisson Labs® culture medium was purchased. The remaining products (sodium hypochlorite, ethanol, Kraft paper, aluminum foil, sanitary and cleaning products, among others) were purchased in local markets. The calculation of the personnel salary was based on the number of hours worked and the Mexican salary tabulator as of January 2021. The electricity, water, and fuel costs were calculated according to their use in the production cycles. Building and infrastructure were not considered, since the study was carried out in functioning laboratories; however, equipment depreciation was considered and an investment project was carried out for this purpose. Microsoft Excel 2016 was used for all calculations.

RESULTS AND DISCUSSION

Induction of adventitious shoots

After two months of culture, 3% of the explants were eliminated due to contamination, mainly by fungi. Fifty percent of the culture media contaminated by bacteria were saved with 50 ppm of silver nanoparticles. During the first three months, adventitious shoots appeared in the meristems. For the medium changes, the same number of flasks were used and, after four months, the first division of the adventitious shoots took place. Afterwards, they multiplied for four more cycles, until the amount necessary for multiplication in the different systems was reached. Thirty 940-ml TIB were used to place 600 initial shoots and 175 initial explants were used to carry out the propagation projection in TIB. The goal was reached in five cycles of 30 days. In a semi-solid medium, the goal was reached in eleven cycles of 30 days. A 10% contamination was attributed to the handling by the operators (Table 1). For the induction and multiplication stage —before scaling up and obtaining 108,200 adventitious shoots—, an average of 416.1 L of culture medium was used.

Multiplication of adventitious shoots

The number of adventitious shoots produced by organogenic culture was 4 for semi-solid media and 18 for liquid media in TIB. The multiplication goal was 500,000 seedlings; this amount was used for the projections and cost analysis of both systems. For the semi-solid medium, 500,500 seedlings were projected using a 1,925-L medium volume during 11 months. For the TIB, 608,000 seedlings were projected, using a 1,200-L medium volume for 5 months. For both systems, a 10% loss due to contamination and a 10% loss due to acclimatization were considered, in the case of the investment project and the

determination of production costs. Various studies about agaves report multiplication rates. Monja-Mio Kelly *et al.*, (2021) report a maximum multiplication rate of 6.23 shoots/explant, using temporary immersion bioreactors in *Agave angustifolia*. Aguilar-Jiménez *et al.*, (2018) reports a multiplication rate of 14.5 shoots of *Agave potatorum* per explant in semi-solid medium. The results of this work surpass those findings, mainly as a consequence of the protocol and the TIB type used.

Seedling acclimatization

After the multiplication phase in both systems was over, the seedlings were sent directly to acclimatization. Arysta® Raizal 400 was used to induce the root in the acclimatized seedling. A 50:50 mixture of peat moss (Sphagnum Peat Moss-Premier Tech Horticulture) and soil from greenhouses was put into polystyrene trays with 200 cavities. There was a 95% survival rate of seedlings from semi-solid medium and 98% of seedlings from TIB. Domínguez Rosales *et al.* (2008) report that, after 30-45 days of acclimatization in *A. potatorum* seedlings, 73% of the seedlings survived. Monja-Mio *et al.* (2020) reported 73% survival of *A. tequilana* seedlings.

Cost calculation and investment project

Initially, the costs of the culture medium per liter used in both systems were compared (Table 2). The gelling agent represents 48.3% of the total cost of the culture medium; therefore, using only liquid media in the TIB reduced production costs. An investment project was developed to carry out the cost analysis, with a fixed asset of US$57,039.71, a deferred asset of US$500.75, and a working capital of US$48,218.16. Table 3 shows the Calculation Report used to carry out the investment project and establish the financial indicators and the cost of production per plant.

The following results were obtained from the cost analysis and the investment project: the cost of producing *in vitro* seedlings using the semi-solid medium was US$0.16 and using TIB was US$0.09 (both including equipment depreciation). In both systems, the

Table 2. Cost of culture medium and percentage in relation to cost.

Description	Cost per semi-solid liter (US$)	Cost per liquid liter (US$) l	Percentage of total cost (%)
Murashige & Skoog with macronutrients, micronutrients, and vitamins Presentation: 1 kg Brand: CAISSON	0.42	0.42	14.3
Gellex® Gellan Gum. Presentation: 500 g., Brand: CAISSON	1.41	-	48.3
6-Benzyl aminopurine (BAP) 25g., Brand: CASSION	0.03	0.03	1.1
Sugar (Chedraui) 2 kg.	0.05	0.05	1.6
Distilled water	1.02	1.02	34.7
Total cost	2.93	1.52	100

The prices of the products were obtained on 02/09/2021. The exchange rate ($19.97 Mexican pesos per US dollar) for that day was checked with Banco de México.

Table 3. Financial indicators of the investment project for the *in vitro* propagation systems of *Agave potatorum* Zuuc.

INDICATORS	SS	TIB
Net Present Value (NPV)*	US$ 375,551.03	US$ 485,127.20
Internal Rate of Return (IRR)	185.39%	237.03%
Benefit/Cost Ratio (BCR)	2.33	3.75

*The indicators were calculated in Mexican pesos and converted to US dollars at a rate of $19.97 Mexican pesos per US dollar.

seedlings are *in vitro* without acclimatization. A selling price of US$0.5 was established for both systems, which indicates that they have a good profitability. In order to corroborate these findings, the financial indicators of the investment project were calculated for both systems (Table 4). The Benefit/Cost Ratio (BCR) for semi-solid media (SS) and for TIB were 2.33 and 3.75, respectively. For every dollar invested, 1.33 is recovered for the SS

Table 4. Calculation report for the temporary immersion bioreactors (TIB) and Gelled Media (SS) system. Price in US dollars, with exchange rate of $19.97 Mexican pesos per US dollar, quoted on 02/09/2021.

TIB				SS			
CONCEPT	QUANTITY	UNIT PRICE	TOTAL	CONCEPT	QUANTITY	UNIT PRICE	TOTAL
Scalpel #3	5	$23.72	$118.60	Scalpel #3	10	$23.72	$237.21
Scalpel #4	5	$23.72	$118.60	Scalpel #4	10	$23.72	$237.21
Scalpel blades 24 c/100 pcs	10	$11.07	$110.67	Scalpel blades 24 c/100 pcs	20	$38.86	$777.21
Scalpel blades 11 c/100 pcs	10	$11.07	$110.67	Scalpel blades 11 c/100 pcs	20	$38.86	$777.21
Surgical mask c/50 pcs	14	$6.91	$96.75	Surgical mask c/50 pcs	28	$6.91	$193.49
Surgical cap c/100 pcs	7	$14.97	$104.81	Surgical cap c/100 pcs	14	$14.97	$209.61
Gellex® Gellan Gum. Presentation: 500 g Brand: CAISSON (G017-500GM)	1	$82.55	$82.55	Gellex® Gellan Gum. Presentation: 500 g Brand: CAISSON (G017-500GM)	14	$82.55	$1,155.66
Murashige & Skoog with macronutrients, micronutrients, and vitamins Presentation: 100 L Brand: CAISSON (MSP09-100LT)	30	$56.73	$1,702.02	Murashige & Skoog with macronutrients, micronutrients, and vitamins Presentation: 100 L Brand: CAISSON (MSP09-100LT)	22	$56.73	$1,248.15
Benzylaminopurine Brand: Phytotechnology (b800-25g)	2	$78.42	$156.84	Benzylaminopurine Brand: Phytotechnology (b800-25g)	1	$78.42	$78.42
pH Buffer	3	$8.01	$24.04	pH Buffer	3	$8.01	$24.04
Potassium hydroxide 250 g	2	$8.01	$16.02	Potassium hydroxide 250 g	2	$8.01	$16.02
Hydrochloric acid 1 l	1	$6.26	$6.26	Hydrochloric acid 1 l	1	$6.26	$6.26
Consumables	1	$751.13	$751.13	Consumables	1	$751.13	$751.13
Work days	336	$10.02	$3,365.05	Work days	336	$10.02	$3,365.05
TOTAL			$6,763.99	TOTAL			$9,076.65

system and 2.75 for the TIB system. This proves the profitability of micropropagation. Likewise, the use of TIB —in addition to improving plant quality— increases at least two times the rate of multiplication and decreases time, vastly increasing the profitability of this type of multiplication. The use of biotechnological methods to decrease the cost of massive multiplication does not only promote the creation of technology-based companies, but also makes these technologies accessible to producers, meeting the demand for this species of agave used in the production of mezcal and helping to prevent the decline of wild populations of the Tobalá agave.

CONCLUSIONS

The use of biotechnological tools becomes more common day by day. Strategies have been developed for the transfer and adoption of plant micropropagation technologies. However, plant tissue culture laboratories are still preferred over biofactories. The difference between laboratories and biofactories is that the latter already use validated protocols and focus on plant quality, as well as the efficiency and (above all) the profitability of the processes. Consequently, works such as ours are required to start the transfer of technologies and encourage their adoption by entrepreneurs or by already established companies. This study was able to demonstrate that both processes have good profitability; however, the use of TIB exceeds profitability by almost 1.5 times, demonstrating that the use of this method in this agave species can have a positive impact on the adoption of the technology by the companies. This method is the first proposed for this species; therefore, it is innovative and can be used for transfer to different entities. A large amount of raw material can be exploited in other market niches —*e.g.*, ornamentals, biomass production, sugars, etc.

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